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Original Research Article

Simple Determination of Inulin Polymerization Degree Average from Dahlia Tuber Using Spectrophotometer

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ABSTRACT

Degree of polymerization (DP) of inulin is the number of monomer units in inulin polymer strand. DP of inulin can be determined simply using spectrophotometer. This study aims to determine DP of inulin from fresh dahlia tubers and dahlia tubers that stored for fifteen days. Inulin extraction from dahlia tuber was based on the solubility of inulin in hot water and precipitation in ethanol by adding ethanol to final concentration of 80% solution. Reducing sugars of inulin was determined using the DNS method. The absorbances were measured using spectrophotometer at maximum wavelength of 490 nm. Total sugar of inulin was determined using Phenol-sulphate method, the absorbance was measured at λ_{max} 350 nm. Inulin from fresh dahlia tubers had higher DP than inulin from dahlia tuber that stored for fifteen days. Inulin that found in this research belongs to the fructooligosaccharide (FOS) group.

***Keywords*:** *Degree of polymerization (DP), dahlia tubers, DNS, phenol-sulphate, reducing sugars, spectrophotometer.*

INTRODUCTION

Inulin is fructant group polysaccharide, which consists of the main unit β-(2→1) fructofuranosil (Fm) and one α-glycopiranose (1→2) (GFn) terminal unit. The DP of inulin varies from 2 to 70, whereas inulin molecules with DP 2-10 are called oligofructose or fructooligosaccharides (FOS) (Petkova & Denev, 2015).

Natural sources of inulin include chicory roots, Jerusalem artichokes, dahlia tuber, yacon, asparagus, leeks, onions, bananas, wheat and garlic (Shoaib et al., 2016). Most commercial inulin is produced from chicory, dahlia tubers and Jerusalem artichoke because it has high inulin content.

Inulin is widely used in the food industry to modify textures, replace fat or as low-calorie sweetener. Generally, inulin is used as prebiotic for the development of functional foods to improve health. In addition, it has several applications in other fields such as pharmacy. Mostly inulin is used as diagnostic agents for kidney function and as protein stabilizer (Mensink et al., 2015; Shoaib et al., 2016).

DP of inulin has effect on inulin function (Shoaib et al., 2016). Inulin with low DP is suitable to be used as prebiotic, whereas inulin with a high DP can be hydrolyzed into FOS. In this regard, the development of analytical methods for determining of DP of inulin is very important for the inulin characterization and its derivatives in plant samples (Saengkanuk et al., 2011). Some simple methods for determining of DP of inulin are fast, specific and suitable for analysis

including spectrophotometry, and chromatographic methods (Petkova and Denev, 2015).

The inulin DP is influenced by several factors, such as plant sources, climate and growth conditions, maturity of the harvest and storage time after harvest (Chi et al., 2011). In this study, inulin DP was determined from fresh dahlia tubers and dahlia tubers which are stored for fifteen days.

RESEARCH METHODS

Time and Place

This research was done in the Chemistry Laboratory, Faculty of Mathematics and Natural Sciences, Universitas Negeri Padang from February to July 2019.

Tools and Materials

Tools

The tools used in the research carried out were blender, sifter 850 µm, glassware, refrigerator, technical balance and analytical balance, centrifugation, filter cloths, oven, thermometer, micro pipettes, micro tubes, water bath, spectrophotometer genesys 20.

Materials

Materials that needed in the research were dahlia tubers, citric acid 0.5%, ethanol, aquades, DNS reagents, fructose standard solution, phenol 5%, and H_2SO_4.

Research Procedure

Inulin Extraction from Dahlia Tubers

Inulin extraction from fresh dahlia tubers and dahlia tubers stored for fifteen days was carried out according to the procedure K. Khuenpet et al. (2016) and Bang-orn Srinameb et al. (2015) which was slightly modified. Dahlia tubers were washed clean, cut in half, one piece was stored for fifteen days and the other piece was skinned, cut into small pieces. Pieces of dahlia tubers were immersed in citric acid 0.5% for 5 minutes, then heated in water bath 75°C for 2 minutes. The slice was cooled and then dried in oven at 55°C for 7 hours. The dried pieces were mashed and then sieved using sieve 850 µm.

Inulin from dahlia tuber was extracted in hot water at 70°C for 30 minutes with a water: flour ratio of 10:1. The extract was filtered while warm with cloth filter. The filtrate was added with ethanol to final concentration of 80% solution, then stored in the refrigerator at 2°C for 19 hours, then allowed to stand at room temperature for 2 hours, then centrifuged at 5000 rpm for 15 minutes at 4°C to obtain a precipitate and supernatant. The precipitate was dried in oven at 40°C.

Fructose Standard Curve of DNS Method

DNS reagents were made according to the procedure of Coughlan & Moloney (1988). Standard fructose solution was made with a concentration of 200-600 µg/mL. Each fructose solution was pipetted 75 µL into a micro tube, then added 75 µL of DNS reagent. The solution was heated in boiling water for 10 minutes, cooled at room temperature, added 850 µL distilled water and homogenized. Absorption was measured at λ 490 nm.

Preparation of the Fructose Standard Curve of the Phenol-Sulphate Method

Concentrations of fructose standard solution were 200-800 µg/mL. Each fructose solution was pipetted 1 mL into a test tube, then added 0.5 mL of phenol 5%, shaken, then added 2.5 mL of H_2SO_4 p.a by pouring perpendicularly to the surface of the solution quickly. The solution was left for 10 minutes, then shaken. The solution was placed in a water bath for 15 minutes, then cooled at room temperature, then added 8 mL of distilled water, and homogenized. Absorption was measured at λ 350 nm.

Determination of Reducing Sugar

Inulin 1% was pipetted 75 µL into a micro tube, then added 75 µL DNS reagent. The solution is placed in a boiling water bath for 10 minutes, and cooled at room temperature. The solution is diluted with distilled water and homogenized. Absorption was measured at λ 490 nm.

Determination of Total Sugar

Inulin 1% was pipetted 1 mL into the test tube, then added 0.5 mL of phenol 5%, shaken, then added 2.5 mL H_2SO_4 p.a by pouring perpendicularly to the surface of the solution quickly, allowed to stand for 10 minutes, then shaken. The solution was placed in a water bath for 15 minutes, then cooled to room temperature. The solution was diluted with distilled water and homogenized. Absorption was measured at λ 350 nm.

Calculation of the Inulin DP

Average of inulin DP was calculated based on the total sugar content per reducing sugar content (Saengkanuk et al., 2011).

RESULTS AND DISCUSSION

Inulin Extraction from Dahlia Tubers

Inulin extraction from dahlia tubers is based on the solubility of inulin in hot water and precipitation with ethanol at low temperatures. Dahlia tubers were washed, skinned and cut into small pieces to speed up the drying process. The process of soaking tubers in citric acid 0.5% aims to prevent browning reactions in the tubers. The browning process in fruit that occurs due to enzymatic processes. The drying process aims to reduce the water content in the tubers. The milling and sifting process is carried out in order to obtain finer root flour so as to facilitate the inulin extraction process.

Inulin from dahlia tuber was extracted in hot water at 70°C for 30 minutes to dissolve the inulin in the dahlia tuber. The process based on the solubility of inulin in hot water. Temperature above 135°C will damaged inulin structure. The inulin filtrate in cold ethanol 80% was centrifuged at 5000 rpm for 15 minutes, 4°C. The precipitate was dried. Inulin that obtained from dry weight dahlia tuber of fresh tuber was 22.2% while inulin of dahlia tuber from fifteen days storage was 23.3%. This difference is caused by decrease in water content during the storage time.

Fructose Standard Curve of the DNS Method

Absorbance of fructose standard solutions was measured at a concentration of 200 to 600 µg/mL at λ 490 nm. The regression equation obtained is y = 0.0018x + 0.1608 with an R^2 value of 0.9917. This value showed that the absorbance at each concentration of fructose solution is very close to a straight line. The curve of fructose concentration and its absorbance was shown in Figure 1.

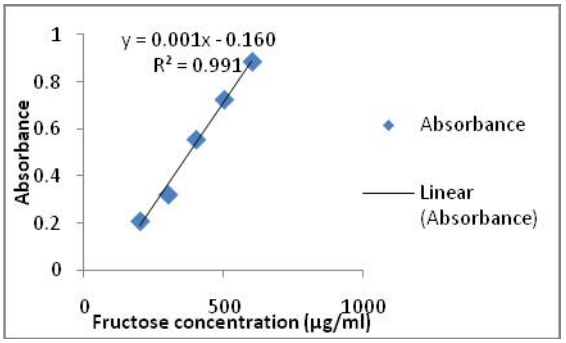

Fig. 1. Fructose Standard Curve using DNS Reagent

The linear regression equation of the standard fructose solution using DNS reagent was used to determine the reducing sugar in inulin.

The Standard Fructose Curve of the Phenol-Sulphate Method

Absorbance of standard fructose solutions was measured at a concentration of 200 to 800 µg/mL at λ 350 nm. The regression equation obtained is y = 0.0011x + 0.0736 with an R^2 value of 0.9904. This absorbance shows that each concentration of fructose solution is very close to a straight line. The curve of fructose concentration and its absorbance was shown in Figure 2.

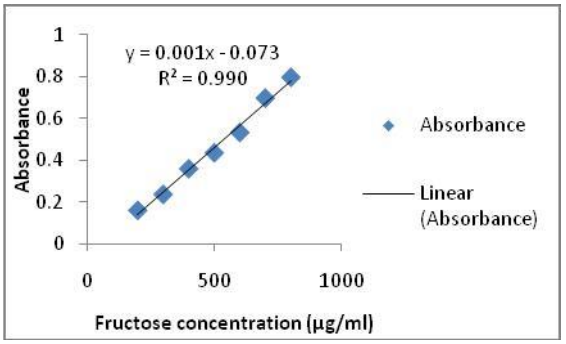

Fig. 2. Fructose Standard Curve using Phenol-Sulphate

The linear regression equation of the standard fructose solution using phenol-sulphate is used to determine the total sugar in inulin.

Reducing Sugar Contents

The characteristic of reducing sugars is the ability to reduce them in an alkaline state. All monosaccharides are included in the reducing sugar group. Disaccharides which include reducing sugars are maltose, lactose, and isomaltose. The sugar has reducing properties because sugar molecule had ketones or aldehydes group (Goutara and Widjandi, 1975).

Reducing sugars in inulin was determined using dinitrosalicylic acid (DNS) method. The value of reducing sugar of inulin obtained in fresh tubers was 8.020 µg/mL, while the value of reducing sugar obtained in stored dahlia tubers for fifteen days was 8.264,44 µg/mL.

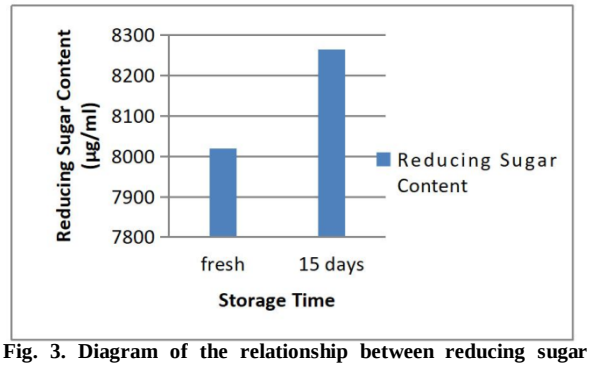

Fig. 3. Diagram of the relationship between reducing sugar content and storage time of dahlia tubers.

Value of inulin reducing sugars in the dahlia tubers increase with storage time. Value of inulin reducing sugars in stored dahlia tubers for fifteen days was greater than inulin in fresh dahlia tubers. Dahlia tuber contained inulinase. The enzyme can catalysis hydrolysis reaction of inulin to produce fructooligosaccharides (FOS) through endo-inulinase activity, and become fructose monomers through exo-inulinase activity. Exo-inulinase can produce fructose by cutting the β-2.1 bond of inulin molecule (Magunwidjaja et al., 2014). The more inulin that hydrolyzed, the more fructose and FOS are formed so that the greater the amount of reducing sugar produced.

Total Sugar Contents

Total sugar or also called total carbohydrate according to Apriyantono et al (1986) is the sum of all simple sugars, oligosaccharides, polysaccharides and their derivatives. Analysis to determine the total sugar is done by the phenol-sulphate method. The sample was reacted with phenol in H_2SO_4 p.a to produce a stable orange-yellowish color. Total sugar content was calculated from absorbance values obtained based on the standard curve that equation (Figure 2), so that the total sugar content in fresh samples was 41.780 µg/mL and the total sugar content in fifteen days stored samples was 21.451,81 µg/mL.

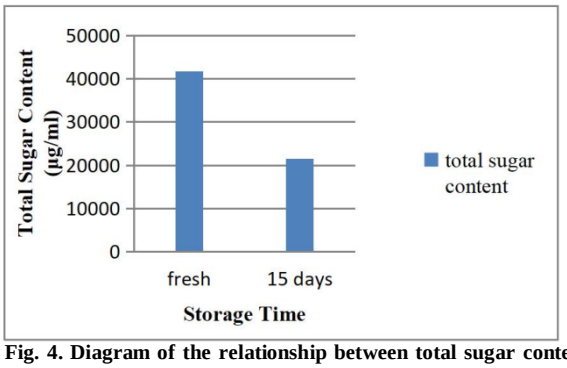

Fig. 4. Diagram of the relationship between total sugar content and storage time of dahlia tubers.

The total sugar contained in inulin from fresh dahlia tubers is higher than the total sugar contained in inulin from dahlia tubers stored for fifteen days. Total sugar was determined based on complete hydrolysis of inulin using acids. Product of the complete hydrolysis was fructoses and glucoses. Process hydrolysis of inulin that had high DP produce more fructose monomers, so its contain high total sugar. Total sugar was determined for determination of inulin DP in dahlia tubers.

Polymerization Degree of Inulin

Inulin DP is the number of monomer units in an inulin polymer strand. DP of inulin depends on many factors, such as plant sources, climate and growth conditions, maturity of the harvest and storage time after harvest (Chi et al., 2011).

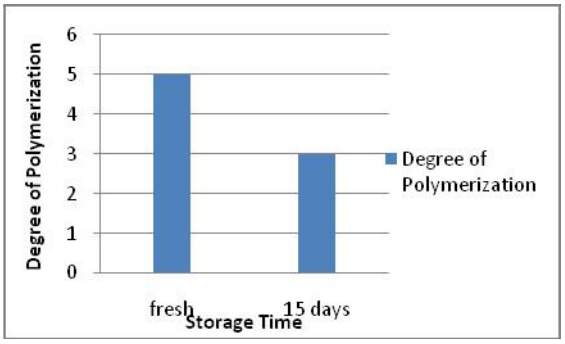

Fig. 5. Diagram of the relationship between the degree of polymerization and the storage time of the dahlia tubers

The storage time has effect on inulin DP of dahlia tubers (fig. 5). Inulin DP from fresh dahlia tubers higher than inulin DP in stored dahlia tubers fifteen days. Dahlia tuber contained inulinase. Inulin DP from freshly extracted dahlia tubers is greater than dahlias which are stored for 4 weeks (Hevi, et al., 2017). The same thing in the artichoke storage period accompanied a decrease in average of inulin DP level, which was caused by depolymerization of high molecular weight carbohydrate molecules (Chi et al., 2011). Inulin DP in Jerussalem artichoke tubers changes during storage after harvest. Inulin DP fraction of 3-10 increased and the inulin DP fraction >10 decreased after 4-6 weeks of storage of Jerussalem artichoke tubers (*Helianthus tuberosus L*) (Saengthongpinit, 2005).

Inulinase is an enzyme that is able to hydrolyze inulin to form fructooligo-saccharides (FOS) through endo-inulinase activity, and become fructose monomers through exo-inulinase activity. Exo-inulinase can produce fructose by cutting the β-2.1 bond in sequence. Endo-inulinase can produce FOS by randomly cutting and hydrolyzing the internal bonds in inulin (Magunwidjaja et al., 2014). The inulin was hydrolyzed to produce FOS (lower inulin DP) and fructose. Inulin DP contained in fresh dahlia tubers was on average 5 (fructosylnystose), whereas DP of inulin on dahlia tubers stored for fifteen days was average 3 (kestose). Inulin extracted from stored dahlia tubers causes DP to be smaller.

The inulin DP from dahlia tubers is quite low, including the FOS group. This is also possible because the extracted sample of tubers is still young. DP has an effect on inulin function (Shoaib et al., 2016). Inulin with low DP is suitable for alcoholic fermentation, or for FOS production. FOS is commonly used as a substitute sweetener for sucrose in products such as cakes, bread, sweets, milk products, and some drinks because it is low in calories.

CONCLUSION

Based on the results of the study it can be concluded that inulin DP from freshly extracted dahlia tubers has an average DP higher than inulin from tubers extracted after fifteen days.

ACKNOWLEDGMENT

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REFERENCES

Apriyantono, A., D.Fardiaz, N.L. Puspitasari, Sedarnawati and S.Budiyanto. 1988. *Food Analysis*. Bogor Agricultural University (IPB Press), Bogor.

Chi, Z. M. Z. M., Zhang, T., Cao, T. S., Liu, X. Y., Cui, W., & Zhao, C. H. 2011. Biotechnological potential of inulin for bioprocesses. *Bioresource Technology*. Elsevier Ltd, 102 (6), pp. 4295-4303.

Horiza, H., Azhar, M., & Efendi, J .. 2017. Extraction and Characterization of Inulin from Dahlia Tubers (Dahlia sp.L) Fresh and Stored. *Eksakta UNP*. Vol. 18 no.1.

Khuenpet, K., Jittanit, W., Sirisansaneeyakul, S., & Srichamnong, W., 2016. Inulin Powder Production from Jerusalem Artichoke (Helianthus tuberosus L.) Tuber Powder and Its Application to Commercial Food Products. *Journal of ISSN Food Processing and Preservation* 1745-4549.

Mangunwidjadja, Djumali, Mulyorini Rahayuningsih, and Reni Suparwati. 2014. *Effect of Enzymatic Concentration and Enzymatic Hydrolysis Time on the Quality of Fructo-oligosaccharides from Inulin Dahlia Tubers (Dahlia pinnata)*. Bogor: IPB, Vol: 3 No.1.

Mensink, M. A. Frijlink, H. W., Van Der Voort Maarschalk, K., & Hinrichs, W. L. J. 2015.

Inulin,a flexible oligosaccharide I:Review of its physicochemical characteristics. *Carbohydrate Polymers*. Elsevier Ltd., 130, pp. 405–419.

Petkova, N., & Denev, P. 2015. Methods for Determination of Inulin. *Monograph of 4rd European Young Engineers Conference 2015*, (ISSN 2367-6213), 135-140.

Saengthongpinit, W., Sajjaanantakul. 2005. Influence of Harvest Time and Temperature Temperature on Characteristics of Inulin from Jerusalem artichoke (Helianthus tuberosus L.) Tubers. *Postharvest Biology and Technology*. Volume 37: 93-100.

Shoaib, M., Shehzad, A., Omar, M., Rakha, A., Raza, H., Sharif, H. R., Niazi, S. 2016. Inulin: Properties, health benefits and food applications. *Carbohydrate Polymers*, 147, 444–454.

Srinameb, B., Nuchadomrong, S., Jogloy, S., Patanothai, A., Srijaranai, S., 2015. Preparation of Inulin Powder from Jerusalem Artichoke (Helianthus tuberosus L.) Tuber. *Plant Food Hum Nutr*. Spinger Science + Business Media New York. Published online: March 25 2015.

Wijandi, S and Goutara. 1975. *Basic Sugar Processing I.* Department of Agricultural Product Technology - FATEMETA IPB. Bogor.

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